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PRELIMINARY NOTES ON THE COLORIMETRIC ESTIMATION OF MINUTE QUANTITIES OF LEAD, COPPER, TIN, AND IRON.

By Edward Russell Budden and Herbert Hardy.

(*Read at the Meeting, April* 4, 1894.)

We frequently have occasion to examine samples of liquids containing traces of metals derived from impurities in the ingredients employed in their manufacture, or now more frequently arising from solution of metallic surfaces of machinery with which the liquids come in contact in process of preparation. We may say that in the latter case we find strong evidence in support of our view that the nature and causes of such metallic contamination have hitherto not received sufficient consideration at the hands of many well-known analysts, and have, indeed, in our opinion been in several important cases (of course, quite unintentionally) mis-stated.

There can be very little doubt that some modified gravimetric process would be preferable in many cases of examination of samples of mineral waters and allied aerated beverages. A process of this nature was suggested and carried out with admirable results some time since in cases in which we had the privilege to be associated with Mr. Otto Hehner. But it frequently happens that the analyst is compelled by smallness of sample, or other equally potent reasons, to adopt some process involving a smaller expenditure of material and time. We may, perhaps, be allowed to say incidentally that in our opinion the *time* occupied in commercial analysis does not always receive its due recognition, and it seems very desirable that there should be more uniformity in fees for different classes of work. But, be that as it may, as a matter of fact it is often necessary to employ some form of volumetric process.

The method most frequently adopted appears to be that originally suggested by Miller, in which the solution (rendered acid, or alkaline, according to the class of metal required to be estimated) has a certain quantity of sulphuretted hydrogen in aqueous solution added, and is then compared with a solution containing a known quantity of the same metal similarly treated. The process is, of course, closely comparable with the Nessler test, familiar to every water-analyst, and it gives fairly good results if only one metal be present. In many laboratories a very small quantity of ammonium sulphide solution is used in place of the sulphuretted hydrogen solution, the test being made in the manner suggested by Wanklyn.

Although we find that this method possesses some advantages (in the case of water-analysis, for example), we have, nevertheless, found many practical disadvantages attending its use in some other classes of work, notably in the case of

liquids possessing a slight yellow tinge in the first instance. And, further, we find no little difficulty in attaining uniform tint in the porcelain dishes in which this test is usually conducted.

We have, therefore, adopted a form of Nessler glass for tests of this description, and invariably use freshly-prepared sulphuretted hydrogen solution. We also find it almost essential for obtaining really accurate results to adopt some form of screen which allows white light to pass vertically only through the Nessler cylinder. We had some difficulty at first in obtaining glasses of uniform colour. But by selecting (by kind permission of a firm of manufacturers) glasses from a large stock, we obtained some few sufficiently good for ordinary purposes.

As we found that some curiously contradictory results were occasionally obtained, we were led to note carefully the conditions under which uniformity was possible. Of course, such results would only be noticed by those whose position brought into the laboratory a very large number of samples of one class of goods.

As the result of these observations, we thought it desirable to make a large number of experiments under varying conditions, and we have the honour to lay before the society a very brief epitome of some of the results obtained, in the hope that new suggestions may be made, and better methods of work suggested. For papers are only of value when they induce fresh investigation and improve our common work. If it be wished, we can give some of our work in greater detail, but it seems to us undesirable to over-burden the journal with excessive detail, or to make papers for the purpose of attracting attention to individual workers.

For purposes of comparison, we prepared standard solutions of such strength that 1 cubic centimetre contained 0·0001 gramme of metal. The method of making the test was as follows :

An empirical solution of any given metal was made, and a certain amount added to filtered distilled water in a Nessler tube, and made up to exactly 50 c.c. with similar water; then a few drops of acetic acid were added, and finally about 2·5 c.c. of a fresh saturated solution of sulphuretted hydrogen in water.

The same process was carried out with the substitution of the standard metal solution (in the proportion requisite for giving a precisely similar tint) for the empirical metallic solution, and the strength of the latter thus determined.

But in the case of the standard solution, the sulphuretted hydrogen solution was sometimes added to the water before the addition of the metallic solution, and at other times the metallic solution was added before the introduction of the sulphuretted hydrogen. It was found that a very important difference arose in the two different orders of addition. For instance, in the experiments with an unknown quantity of lead acidulated with acetic acid, to which sulphuretted hydrogen was *subsequently* added, it was found that when the attempt was made to produce the same tint by adding the standard lead solution to the water *already* mixed with the requisite amount of sulphuretted hydrogen the difference amounted to 0·1 c.c. and upwards in the quantity required. Though it was found that in the case of very dilute lead solutions fairly uniform results could be obtained.

Experiments of a similar character with copper gave also very discordant results. For instance, in three successive tests 3·5 c.c., 4·0 c.c., and 4·2 c.c. were

respectively used to secure similarity of tint, giving a difference of 0·7 c.c. between extremes, arising from difference of order of addition of reagents.

We next passed on to experiments with mixtures of metals, since it often happens that several metals are present in fluids with which we are practically concerned. We may mention at this point the value which we found in Dr. Teed's suggestion (made in a paper read before the society some time since), that the coloration, due to the presence of copper, might be prevented by addition of potassium cyanide, while, when iron also is present, its influence on colour reactions may be avoided by the employment of tartaric acid. It is, perhaps, hardly needful to say that this latter must be absolutely pure, and that the tests must be made in alkaline solution.

Certain difficulties, however, arise when other metals are present, to which we shall have occasion to refer subsequently, although Dr. Teed's method possesses great value in many cases.

For experiments on mixed metals we employed solutions containing varying proportions of standard metallic solutions, as we had reason to believe that difficulty would arise in the direction of tints of matching solutions. For it should be noted that in the case, for instance, of lead and copper the tint given in each case by the addition of sulphuretted hydrogen differs widely. In fact, it is practically impossible to carry out the volumetric process with mixtures of some metals on account of the complexity of tints.

We first experimented with copper and lead in the following way: Taking a mixture of equal quantities of solutions containing approximately the same amount of lead and copper, we found it impossible to match the colour accurately either with standard copper or standard lead solution alone, though some approximate estimate could be formed of the amount of metal present. When copper was kept in solution, however, by addition of potassium cyanide, and the mixture matched by standard lead solution alone (still adding the metal after the introduction of the sulphuretted hydrogen, as in the case of our experiments with single metals), we found in some cases an error amounting to 0·2 c.c. of standard metal solution, or about 20 per cent.

Thus it will be seen that adding the standard metallic solution after the sulphuretted hydrogen to the matching solution in the Nessler cylinder is a method capable of introducing most serious errors into the determination. The nature of the difficulty is apparently indicated by our observation that on using rather stronger solutions than we usually employed, we noticed the production of a minute film of metallic sulphide upon the surface of the liquid as the metallic solution was run in from the burette. This effect is specially noticeable in the case of lead. The production of this film, of course, has the effect of causing more standard metallic solution to be employed than is actually required in any given experiment. For example, in one experiment we recorded an error of 2 c.c. (6 c.c. required in place of 4 c.c.), which equals an experimental error of 50 per cent. of the actual amount of metal present. We also observe that the error increases materially with stronger solutions.

A mixture of lead and copper can be better estimated by employing for com-

parison a lead solution than one of copper, while in this case the addition of potassium cyanide appears to cause too little lead to be used for matching if the metal be added to the solution after the sulphuretted hydrogen. Though in this case the error does not exceed 10 or 20 per cent., yet such an amount seriously impairs accuracy.

It does not appear to make any difference whether lead be compared in acid or alkaline solutions if no other metal be present.

For estimation of mixtures of lead and copper we tried compound matching solutions containing the two metals in equal and other proportions, but found but little success in this direction. Although the difference in colour between the tubes was slight, it was very difficult to judge correctly, while if the proportions of metals are very unequal the difficulty is much increased. If the metal be added before the sulphuretted hydrogen, there is no difficulty in obtaining closely concordant results in the case of copper alone, lead alone, or a mixture of lead and copper. In the case of a mixture of this kind, if potassium cyanide be added to keep up the copper, and the sulphide tint be matched with standard lead, and in a following experiment (on a similar quantity of liquid) an amount of standard lead be first added equal to that used in the first instance, and the difference again matched, using this time standard copper, very accurate results may be obtained, provided always that the metal be added before the addition of sulphuretted hydrogen, otherwise altogether conflicting results are obtained, which are entirely worthless.

It seems to be essential that all experiments with this method must be made under strictly comparable conditions. The same amount of each reagent must be used in each case, and the liquid should be well stirred after each reagent has been added.

The results seem to be most correct when the sample is of the same strength so far as metal is concerned, as the standard metallic solution.

There is a slight error noticeable if much difference exists between the strengths of the solutions, but this error is too small to affect general accuracy of results in practice. If iron be present, it may be prevented from interfering with the tint reactions by the addition of lead-free tartaric acid (as suggested by Dr. Teed).

In those cases where potassium cyanide has also been added to keep the copper in solution, there may be formed a precipitate of Prussian blue if much iron be present. In such an event it would probably be better to estimate the iron by one of the less delicate ordinary volumetric methods.

Thus we have indicated how copper and iron can be practically eliminated from the colour-test and lead estimated in the presence of these metals with considerable accuracy. But in almost every sample of artificial mineral waters, we find that a very notable proportion of tin is discernible. The reason for this and other contamination we shall speak of presently.

We find that by the use of peroxide of hydrogen, tin (if present in small quantities only) can be completely and rapidly oxidized, and will then not perceptibly affect the colorimetric method. But the test must be made rapidly, since after about a minute there is a slight colour perceptible, especially if the amount be somewhat larger than usual; and the colour speedily deepens, and after about five minutes the solution becomes opaque.

An amount of tin that in the stannous condition gives with sulphuretted hydrogen a blackness that makes it impossible to see through two inches of solution, will, if oxidized with peroxide of hydrogen, give, on addition of sulphuretted hydrogen, no more colour than can be matched by less than 0·1 c.c. of standard lead solution. But it becomes opalescent after a very short time.

A standard stannous solution (·0001 gramme tin per c.c.) may be employed for matching the tint accurately, but this will not keep for more than two days. When tin is present as well as lead or copper, the matching with lead or copper or a mixture of both becomes impossible. Therefore the tin must be prevented from affecting the results in some manner such as we have suggested; otherwise large errors of analysis will arise. It therefore seems to us possible to test accurately, on the lines we have indicated, a complex mixture of the four metals most commonly found in combination. But we find that to give correct and comparable results, all tests must be made under similar conditions. The reagents must be added in the same proportion and same order. The sulphuretted hydrogen solution must be added after the addition of metallic solution in the comparison cylinder, otherwise a scum of sulphide is formed seriously affecting results. It may affect them to the extent of 50 per cent. Also, if the metal be added after the sulphuretted hydrogen, a clear tint cannot readily be obtained, while if the addition be made in the proper order, a clear and readily-matched tint is obtained.

The use of potassium cyanide appears sometimes to give rise to complications, owing to the formation of complex and unstable cyanogen compounds, especially in solutions containing iron. It also seems probable that when a large proportion of copper is present a portion may be precipitated after the addition of potassium cyanide. Possibly this may be due to the formation of a cupro-potassium cyanide in which a portion of the metal is capable of precipitation as sulphide.

Peroxide of hydrogen completely destroys the colour produced by some of the metallic sulphides, even when the tints are very strong. But there is no sharp transition from the oxidation of one sulphide to that of another, though the changes appear to take place in some definite order. Yet the stages are too transient to admit of any satisfactory conclusion being drawn from noting the progress of this reaction, otherwise some valuable information as to the relative proportion of the metallic impurities might possibly have been deduced.

In the case of aërated beverages there is always present a notable amount of carbonic acid gas. It therefore seemed desirable to ascertain if any marked effects were produced upon the tints formed in the reactions employed for determining traces of metallic contamination. Experiments were made, therefore, on this point with rather curious results, which may tend to explain certain anomalies hitherto unaccounted for. The gas was in some instances passed through the solutions before the addition of reagents, and sometimes afterwards. Stated briefly, the experiments hitherto made in this direction show that the iron reactions are most influenced by the presence of carbonic acid; that iron is the only metal affected when the gas is passed first, *i.e.*, before any colour-reaction is produced, and this has an important bearing on the testing of mineral waters. Further, it would seem that copper is never affected by the gas. When the colour reaction has taken place with lead and tin,

the passing of carbonic acid gas causes the sulphides to assume the form of a precipitate.

In a paper of great value read before the Society of Chemical Industry by Mr. R. Warington, some observations were made on the employment of glycerol in connection with the sulphide tests for lead in tartaric and citric acids. We therefore made a few experiments to judge of the effect of this addition in the case of other metals, and obtained one or two results of interest.

The addition of a small amount (two or three drops) of glycerin partially prevents the formation of a colour or precipitate in the case of all the metals. The employment of 5 c.c. in 50 c.c. of solution does not materially increase this effect, while if a volume of glycerine equal to that of the tested solution be employed, the precipitation is entirely prevented, even in the case of iron, which under the ordinary conditions obtaining in these experiments was found to precipitate most readily. It seems probable that this curious effect of glycerin is due to mechanical rather than chemical causes, but it would seem to indicate the necessity of tests being made in solutions of similar density if really accurate results are desired.

As the result of experiments and observations hitherto made, we deduce the following conclusions:

That it is above all necessary in determination of traces of metals made by these volumetric processes to follow strictly the same order in the addition of reagents, and as closely as possible to employ always the same quantity of reagent, both in the actual experiment and in the preparation of the solution used for the comparative determination.

That all the conditions of experiment must be similar, for example, as to the state of oxidation of the metal, and the presence of extraneous factors affecting reactions, as in the case of carbonic acid gas or substances affecting specific gravity of samples tested. It would seem that, if our results are confirmed, these experiments carry the method of testing for metallic impurities in solution somewhat further than has hitherto been possible, but we are still hopeful of working out a more completely successful process of differential volumetric testing in the case of solutions containing several metals. If this can be accomplished, much facility will be afforded to the analyst who desires to obtain quickly and accurately an estimation of the proportions of metals causing the aggregate metallic contamination of the beverage or solution submitted for examination.

We may observe that in the case of aërated waters and similar beverages artificially prepared, the predominant metal appears in all cases to be tin. We are speaking, of course, of the products of well-appointed manufactories, equipped with modern machinery, and in which only pure acids are employed. Formerly, when it was practically impossible to obtain tartaric and citric acids free from lead, that metal was frequently found in lemonade and ginger-beer, and other acidulated beverages; while many cases have been recorded, usually from small and imperfectly equipped factories, where a serious amount of lead or copper contamination arose from the use of unsuitable pipes or other appliances. Mr. Stokes showed one of us a sample of ginger-beer from such a source containing a very considerable amount of lead (about 4·5 grains to the gallon). And sometimes contamination has arisen from

the glazing of stoneware bottles and other appliances, such as enamelled metal pans used sometimes for syrup-making. Copper also finds its way from machinery which has not been perfectly coated with some non-injurious material; and there is reason to believe that in some cases, at any rate, a very appreciable amount of copper is dissolved in the aërating cylinders by an electrolytic action set up by the dissolved carbonic acid coming into contact with the tin lining of the vessels and portions of copper exposed by continuous friction. Indeed, we have found a marked increase of copper dissolved when a cylinder has been (by request) allowed to remain filled with carbonated water for an unusually long period. All these points have to be taken into consideration when forming an opinion as to the cause and nature of metallic contamination of beverages. But the most important matter for the analyst is usually to be able to rapidly and accurately estimate the extent of total metallic impurity, and when the quantity is at all considerable, to have a ready method of determining the nature and proportion of the metals severally concerned in producing such total. If these notes tend in any degree to clear the ground in this direction, they will not have unduly occupied the time of the society, we trust.

Discussion.

The Chairman (Mr. O. Hehner) invited discussion on the subject of the paper, and said that he hoped that this would lead to some agreement as to a method for testing water for traces of metallic impurities.

Dr. J. Edmunds referred to the pyrological methods introduced by Colonel Ross, and improved by himself, for the detection and estimation of exceedingly minute quantities of metals. These consisted in reducing a portion of the ash to be examined in a bead of pure boric acid in the flame of the blowpipe, and subsequently examining and measuring the spherule of reduced metal, after the bead had been mounted in Canada balsam, under the microscope. By the use of the filar micrometer, the diameter of the spherules might be taken with great accuracy, and the geometric constants—used with the chemical formula and specific gravity of the substance of the spherule—gave its weight to the millionth of a gramme or even less. A spherule from $\frac{1}{1000}$ to $\frac{1}{10000}$ of an inch in diameter was an easy object to deal with by this method. Copper and lead could be distinctly dealt with in this way, but he (Dr. Edmunds) had not been able to satisfy himself that the method was reliable for tin. Lead and some other metals might be got out on a platinum cathode under the slow action of a couple of Daniell's cells, and then dissolved off and dried up to a residue fit for treatment in boric acid.

Mr. Sidney Harvey thought it was imperatively necessary that the same order should be observed in the comparison of the liquids with the trial test in the case of the estimation of small amounts of metal in solution. That is to say, the reagents must be added in the same order, and in the same amounts.

Dr. Frank L. Teed believed that the amount of lead in a water, when it amounted to one-hundredth of a grain to the gallon, could only be determined by some volumetric process. He had listened with great interest to what Dr. Edmunds had said, but he looked upon his method as a qualitative rather than a quantitative

method of analysis. Mr. Budden's remarks were extremely important, more especially as regards the order in which the solutions were added. In a paper on the same subject Mr. Warington had pointed out that citric and tartaric acids had a specific action on the tint of the lead coloration. He had certainly shown that lead and copper assumed different tints according as they were in presence of water only, or of tartaric, or of citric acid. At the time he (Dr. Teed) was working out his process (on which Mr. Warington's was subsequently based), he was stopped by this obstacle, that there was no pure tartaric, and no pure citric acid to be obtained for love or money. He tested a good many samples, but they invariably contained lead. He suggested in his paper that for the separation of copper, lead, and iron in a natural water, pure tartaric acid should be used, but as he had not got any he could not say that he ever did it. Mr. Warington had proved that citric and tartaric acid affected the colour, and Mr. Budden had shown that carbonic acid also affected it. If it was desired to determine the presence of lead in ginger-beer, he believed the only possible way was to ash it first and get rid of the organic matter. He hoped that Mr. Budden would bring before the society more elaborate notes later on, so that there might be some standard of reference in regard to this subject. In his opinion, when it was a question of ·0003 per cent. of lead in a sample, it did not matter whether the result was ·0003 or ·0004.

Mr. E. J. Bevan did not know whether Dr. Teed made it quite clear to what extent Mr. Warington looked upon the presence of citric acid as the cause of error. He (Mr. Bevan) had had occasion to work on a large number of beverages some time ago, and he had some conversation with Mr. Warington on the subject, and that gentleman informed him of the results he intended to bring forward. He (Mr. Bevan) had found that by adding sugar to the solution the apparent amount of lead was increased as much as ten times. It was necessary, in order to make a satisfactory colorimetric test, that the disturbing constituents should be known, and these must be added to the standard solution. Dr. Teed's course of evaporating down and then igniting would get over that difficulty. It was a remarkable thing that an error of 1,000 per cent. could take place if sugar were present.

Mr. Hehner asked Mr. Budden how he used the peroxide of hydrogen. He understood him to say that turbidity very soon manifested itself.

Mr. Budden replied that he added the peroxide of hydrogen and subsequently the sulphuretted hydrogen water.

Mr. Richmond drew attention to the work of Messrs. Picton and Linder (*Jour. Chem. Soc.*, lxi., 114, 137, and 148), which probably contained the whole explanation of the curious differences observed by Mr. Budden. It seemed to him that variations of colour were due, in some cases, to the formation of different hydro-sulphides. Thus, Messrs. Picton and Linder had obtained with copper hydro-sulphides approximating to the formulæ $7CuS,H_2S$; $9CuS,H_2S$; and $2CuS,H_2S$, of which the first, obtained with excess of sulphuretted hydrogen, was soluble. It seemed to him (Mr. Richmond) that the results of Mr. Warington with citric acid, and those of Mr. Bevan with sugar, were explained by the second and third papers of Messrs. Picton and Linder; they found that different physical modifications show different

colours, and the exact state in which they exist depends partly on the other constituents of the solution. Possibly in these cases the sulphides were more in suspension than they would be with water only as a solvent, and the presence of substances such as citric acid and sugar turned the dissolved body out of solution into a condition of suspension, and thus made the tint appear darker; and therefore in testing for these metals the best thing to do would be to have the conditions most favourable for a state of suspension as against a state of solution—that is to say, this condition should be pushed as far as was compatible with the non-production of actual precipitation, in order to develop the maximum coloration. A study of these papers would be found very interesting.

Mr. Hehner said he believed the statement that citric acid added in quantity would make these sulphides more insoluble was inaccurate. His experience was that citric acid had a contrary effect.

Mr. Richmond admitted that Mr. Hehner's remark, that citric acid prevented complete precipitation of lead sulphide, certainly seemed an apparent contradiction to what he had quoted from Messrs. Picton and Linder's work. But he thought that if their work were read carefully, it would be seen that they had shown that there were so many stages between the actual precipitation and true solution that he (Mr. Richmond) did not think it would be entirely in contradiction. He had also found that lead precipitated as sulphide in presence of citric acid when filtered would run through the filter; but, notwithstanding, though the particles could hardly be seen, the sulphide could be separated from the solution fairly easily, and it certainly was in a state of suspension.

Mr. Bevan asked Mr. Budden what the action of glycerin was; did it keep the lead in solution?

Mr. Budden replied that if there was a certain amount of glycerin added precipitation was prevented, and the matter was kept in solution.

Mr. Hehner thought that the deductions to be drawn from the discussion showed that small quantities of these metals, when mixed together, could be quantitatively determined under certain conditions by colour-tests. When they were in simple aqueous solution there was generally no difficulty; but when it was shown that under other conditions an error might be committed, faith in the colorimetric process was shaken. He had refused to make determinations on very minute quantities of the sample; but he had attempted, with more or less success, by taking a large quantity and evaporating to small bulk, to separate the metals. He imagined that it would be impossible to obtain the metals by incineration. The quantities of sugar and the carbon obtained therefrom were very large. One could not expect to incinerate a milligramme of lead with ten thousand times its weight of sugar without a considerable loss occurring. He would like to ask Mr. Budden whether he had ever used a colorimeter, such as Mills', which would obviate diluting or adding more reagent to the liquid. It was very desirable to arrive at some plan of operation for the future.

Mr. Budden said that the reason which prompted him to bring forward his paper was because he found that some manufacturers of aerated beverages were going the round of the London analysts, and were obtaining results from different chemists

which disagreed with each other, and this seemed to him not only a very unsatisfactory state of affairs, but also one which was not calculated to impress the manufacturers (who wanted to know how they were to get rid of errors in manufacture) with a very high estimate of the methods used by analysts. In regard to ginger-beer, he might say that the metal which generally had to be looked for was lead, and when he could get the beverage sufficiently clear the addition of a trace of acetic acid, and then hydrogen sulphide in solution, enabled the colour to be observed with a certain amount of accuracy. He did not mean to say that it was a perfectly accurate method. The colour produced by ammonia when added to ginger-beer would, of course, necessarily interfere very much with the observations, but he did not see that in some cases it was necessary to have this solution alkalized to that extent. The ammonia appeared to react with some resinous matter in the ginger-beer. The point mentioned by Mr. Bevan interested him exceedingly, because it seemed to be almost the converse of the observation he (Mr. Budden) made about carbonic acid. He had had a case of some dozens of ginger-beer sent to him, all manufactured at one making. After standing some time, the contents of the bottles were found to vary materially in the reactions which they gave, and were also found to vary very materially in the amount of gas present. It seemed obvious that if the amount of effervescence was considerable in any given bottle, there must be a very marked difference in the amount of sugar present. It is very probable, indeed, that the sugar had a marked effect in the same way that glycerine had. He had been making some experiments on a form of colorimeter, and he had had an appliance made which might possibly be useful. It was certainly quite probable that the method might be considerably amplified.

THE EXAMINATION OF URINE FOR SMALL QUANTITIES OF SUGAR.

By Alfred H. Allen.

(*Read at the Meeting, June 6th*, 1894.)

The detection and quantitative determination of sugar in typical diabetic urine presents few difficulties, since in such an excretion the proportion of sugar present is very considerable—ranging from 1 to 10 per cent.—and the interfering constituents of the urine are correspondingly reduced in quantity. But when the proportion of sugar is but small, by which I mean below 0·25 per cent., the difficulties attaching to its recognition and accurate determination are considerable, and have not been wholly overcome by any of the numerous investigators of the problem. Under these circumstances, it appeared probable that a review of the present position of the question, supplemented with some observations of my own, would be of interest to the members of the Society.

The question of the occurrence of traces of sugar in normal urine has been the occasion of much controversy. Brücke appears to have been the first to state that all normal urine contained sugar, and this view was supported by Bence Jones and by Kühne, but opposed by Friedlander, Wiederhold, Meissner, and Babo. The question was re-examined in 1871 by Seegen, who pointed out many fallacies in the methods of those who had found sugar in normal human urine, and concluded that it was either

absent, or present in such small proportion that the then existing methods were insufficient for its positive recognition in the presence of co-occurring bodies which simulate many of its reactions. On the other hand, Dr. F. W. Pavy, in 1878, concluded that sugar was a normal constituent of urine, and that no sharp line of demarcation could be drawn between the excretion in health and in diabetes, except quantitatively. Mr. G. Stillingfleet Johnson, again, by an improved method of examination found no trace of sugar in healthy urine, while Molisch, from the examination of a large number of samples of healthy human urine by the alpha-naphthol and thymol tests, came to the conclusion that traces of sugar are met with frequently in human urine; but the value of his tests, and hence the accuracy of his conclusions, have been disputed by Leuken and also by Seegen (*Jour. Soc. Chem. Ind.*, vi., 149, 150). E. Luther, again (*Chem. Centr.*, 1891, ii., 90; and *Jour. Chem. Soc.*, lx., 1559), as the result of the application of the furfuraldehyde and alpha-naphthol tests to a large number of samples, concludes that glucose is present in all human urine, the amount found in the excretion of adults averaging 0·1 per cent., while the total carbohydrates amount to 0·2 per cent. According to E. Roos (*Zeit. Physiol. Chem.*, xv., 513), the normal urine of the dog, horse, and rabbit always contains more or less carbohydrates, as indicated by the furfuraldehyde reaction and confirmed by the benzoic chloride test. Human urine is stated by Roos always to give an affirmative reaction with phenyl-hydrazine, and the same is true of dogs' urine, while the excretion of rabbits gives especially well-formed crystals. The urine of all these animals was found to be slightly lævo-rotatory.

By the production of the crystalline phenyl-glucosazone—a method free from the objections and fallacies which underlie nearly all other tests—I have proved to my complete satisfaction that sensible quantities of sugar are present in some specimens of urine from healthy persons.

The fact appears to be that, while normal human urine may sometimes contain traces of sugar, that substance is by no means constantly present, and a great number of the recorded observations are quite inconclusive.

Passing by the polarimetric and fermentation tests for sugar, neither of which is adapted for the recognition of very small quantities, the tests dependent on the reducing action of glucose are those which are best known and deservedly popular. But it is well known to all who have worked on the subject that these tests often give very disappointing results, even in the hands of experienced analysts, and when employed in the hurried and slovenly way very common in clinical testing give indications which are difficult to interpret or absolutely worthless. These failures, and the false conclusions frequently based on them, are largely due to the interfering action of some of the normal constituents of urine, the presence or influence of which is usually ignored. Hence it is important to consider the extent to which these bodies interfere, and the manner in which they may be removed or their influence obviated.

The chief of these interfering bodies are uric acid, xanthine, and creatinine, but under some conditions urine contains glycuronic acid or compounds thereof which simulate sugar very closely. Hence before the usual tests can be satisfactorily employed for the detection of small quantities of sugar, the above interfering sub-

stances should be removed as far as possible. The amount of uric acid passed per diem under ordinary conditions is said to be about 0·5 gramme, though, of course, in many instances it is considerably more. Xanthine and the allied bodies are present in still smaller amount. Although chemists are in the habit of taking account of uric acid, because it makes itself evident to the senses, they habitually ignore the presence of creatinine. According to Voit, the proportion of creatinine passed in twenty-four hours ranges from 0·5 to nearly 5 grammes. Urine containing the latter amount would exert a reducing action on Fehling's or Pavy's solution equivalent to the presence of 0·32 per cent. of glucose. Hence it is very important to remove these interfering bodies when testing for small quantities of sugar. For this purpose metallic precipitants can be used with considerable success. Thus, for instance, urine can be conveniently clarified and freed from albumen (if present), uric acid, phosphates, and colouring matters, by precipitating boiling hot with neutral lead acetate. Basic lead acetate removes certain other bodies which escape precipitation by the neutral salt, but there is no material advantage in its use. In either case, the filtered liquid is colourless, or very pale, and is well fitted for optical examination, or testing by the phenyl-hydrazine reaction.

Cupric sulphate yields little or no precipitate with normal urine in the cold, but on standing or boiling a whitish or pale-green precipitate is thrown down which has a tendency to darken if the heating be continued. If acetate of copper be substituted for the sulphate, or if sodium acetate be added to the cupric sulphate solution, the precipitation is much more complete, uric acid, xanthine, hypoxanthine, colouring matter, and albumen (if present) being entirely thrown down, and the creatinine and phosphates partially.* The filtered liquid cannot be used for the phenyl-hydrazine test, and the presence of copper unfits it for titration by Pavy's solution; but I have found it admirably suited for the detection of small quantities of sugar by Fehling's test, as follows:

From 7 to 8 c.c. measure of the sample of urine is heated to boiling in a test-tube, and, without separating any precipitate of albumen which may be thrown down, 5 c.c. of the solution of cupric sulphate used for preparing Fehling's test is added. This produces a precipitate containing uric acid, xanthine, hypoxanthine, phosphates, etc. To render the precipitation complete, however, it is desirable to add to the liquid, when partially cooled, from 1 to 2 c.c. of a saturated solution of sodium acetate having a feebly acid reaction. The liquid is next filtered. To the filtrate, which will have a bluish-green colour, 5 c.c. of the alkaline tartrate mixture used for preparing Fehling's solution is next added, and the liquid boiled for fifteen to twenty seconds. In the presence of more than 0·25 per cent. of sugar, separation of cuprous oxide occurs before the boiling-point is reached, but with smaller proportions precipitation takes place during the cooling of the solution, which becomes greenish, opaque, and suddenly deposits cuprous oxide as a fine orange-yellow precipitate. When a urine rich in sugar is under examination, the volume taken can be advantageously reduced from 7 or 8 c.c. to 2 or 3 c.c., or even less, water being added to replace it.

It is evident that in this modification of the ordinary Fehling's test, advantage

* The precipitation of the phosphates of urine by cupric acetate is not complete, but the circumstance has no influence on the detection of glucose in the filtrate.

is taken of the very general precipitating power of cupric acetate, to remove from the urine the great majority of those substances which interfere with the detection of diabetic sugar, by themselves reducing the alkaline copper solution, retaining the cuprous oxide in solution, or producing a flocculent precipitate which masks the true reaction of sugar. Operating as described above, no greenish turbidity refusing to settle is produced, and hence the separation of any cuprous oxide is very readily observed.

It is important that the sodium acetate should not be added till the liquid has partially cooled, so as to avoid any chance of reaction of the resultant cupric acetate with the glucose in the manner observed by Barfoed.

Pavy's method of determining diabetic sugar by titration with ammoniacal cupric solution would probably be more generally applied if it did not necessitate the use of a special apparatus. To avoid this disadvantage I have devised the following form of the test, which is simple and convenient, but less accurate than where larger quantities of the urine and reagent are employed. An accurately-measured volume of 10 c.c. of Pavy's solution is placed in a wide test-tube, a few fragments of tobacco-pipe dropped in, and 8 to 10 drops of petroleum or paraffin burning oil added. This forms an upper layer which effectually excludes the air. The test-tube is inserted into the neck of a wide-mouthed flask containing hot water, which is then heated until the contents of the tube have reached the point of ebullition. The urine to be tested is treated with an equal measure of ammonia, and filtered from the precipitated phosphates. A known volume of the filtrate is then further diluted with a definite measure of water, according to the proportion of sugar supposed to be present, and then added drop by drop to the boiling hot Pavy's solution by means of a small burette or graduated pipette, until the disappearance of the blue colour indicates the termination of the reaction. If 10 c.c. of Pavy's solution was employed, the volume of urine required to decolorize it contains 0·005 gramme of sugar. Or if 100 grain measures of the copper solution were used, the urine contained 0·05 grain of sugar. By operating in the foregoing manner, fair approximate determinations of sugar in diabetic urine are obtainable very rapidly and with the simplest of apparatus.

Experiments in my laboratory by Mr. G. Bernard Brook show that unclarified healthy human urine exerts a reducing action on Pavy's solution equal to that of a liquid containing from 0·1 to 0·3 per cent. of glucose. G. Stillingfleet Johnson finds the reduction to vary from 0·15 to 0·19 gramme per 100 c.c. (= 0·6 to 0·8 grains per fluid ounce), and ascribes about one-fourth of this to uric acid (removable by lead acetate) and the remainder to creatinine (removable by mercuric chloride).

It is evident, therefore, that Pavy's method, applied in the ordinary manner, is apt to give misleading results when only small quantities of sugar are in question. As to Fehling's test, although, by the foregoing modified mode of application, the indications are much more definite, and the delicacy of the reaction is correspondingly increased, there still remains the disturbance due to the presence of creatinine. On adding Fehling's solution to a solution of this substance, a green liquid is produced, and on boiling a yellow coloration is observed, without, however, any separation of cuprous oxide. It is this behaviour which causes interference with the detection of glucose, the combination of the yellow and blue colours resulting in a green, and in

addition the creatinine compound is said to have the power of preventing the precipitation of cuprous oxide by glucose.

A better separation of creatinine can be effected by a method proposed by Maly and improved by G. Stillingfleet Johnson. The latter chemist adds to the urine 25 per cent. of its volume of a cold saturated solution of mercuric chloride and 5 per cent. of a similar solution of sodium acetate. This reagent effects an immediate precipitation of any albuminous matters which may be present, together with the whole of the phosphates, urates, and most of the colouring matters, and the xanthine bases. If the liquid be filtered immediately, the creatinine remains in solution, but is deposited in the course of a few days. It may, however, be completely thrown down by boiling the liquid for a few minutes. In the filtrate thus purified from all interfering bodies, Mr. Johnson determines the sugar by Pavy's ammoniacal cupric solution, after precipitating the excess of mercury.* Sulphuretted hydrogen is not available for this purpose, since it gives rise to certain fixed derivatives which reduce alkaline cupric solutions. Johnson adds ammonia drop by drop, and thus secures perfect precipitation of the mercury, but this plan has not given good results in my hands, and if the mercury be not completely removed at this stage the subsequent operation is vitiated. I have obtained the best results by boiling the liquid for five or ten minutes with zinc-dust, which precipitates the mercury perfectly. Sixty c.c. of the urine to be tested should be boiled for five minutes with 15 c.c. of saturated mercuric chloride and 3 c.c. of saturated sodium acetate solution, and the liquid filtered hot. The precipitate is washed twice, and the filtrate boiled for ten minutes with zinc-dust and again filtered. The precipitate is washed, and the filtrate diluted to 120 c.c. with ammonia (specific gravity 0·960). This liquid has half the concentration of the original urine, and is added to not more than 50 c.c. of boiling Pavy's solution from a burette in the usual manner. The requisite washing of the mercuric and zinc precipitates can be avoided if 50 c.c. of the urine be boiled with solid mercuric chloride and sodium acetate, the liquid filtered, the filtrate boiled with zinc-dust, and again filtered, and a known volume of the last filtrate mixed with an equal measure of strong ammonia.†

In experiments made in my laboratory by Mr. G. Bernard Brook in the foregoing manner, upon urine from apparently healthy persons, the purified liquid exerted a reducing action on Pavy's solution corresponding to the presence of from 0·05 to 0·13 grammes of glucose per 100 c.c. of the original urine, which yielded crystals of phenyl-glucosazone by the phenyl-hydrazine test. I have proved by parallel experiments on the same sample of urine that Johnson's treatment with ammonia (when successful) and mine with zinc-dust, give the same results. In the case of every urine hitherto examined in my laboratory, there has been a slight residual reduction after the

* Since this paper was read, Sir George Johnson (in an interesting paper published in the *Lancet* for June 7, 1894), proposes to eliminate the creatinine, etc., by boiling with mercuric chloride and sodium acetate, in the manner devised by his son, separate the excess of mercury by ammonia, and determine the sugar colorimetrically in the filtrate by boiling with caustic alkali and picric acid. He finds no brown coloration indicative of glucose to be produced by normal urine which has been previously clarified by mercury.

† This treatment serves the double purpose of keeping the zinc in solution and furnishing a constant but gradually added supply of ammonia during the subsequent titration with Pavy's solution. The additional ammonia has been proved by Mr. C. G. Moor to have no prejudical effect on the accuracy of the results obtained.

mercury treatment, and I consider it highly probable that these urines, which were limited in number, contained traces of actual sugar. But Mr. Johnson has obtained entirely negative results, which implies that an infinitely large quantity of the urines he examined could be added to Pavy's solution, after purification by mercury, without causing the slightest reduction in the colour.

As all the methods of detecting sugar in urine which are based on the reducing action of glucose are more or less vitiated by the presence of other reducing bodies, a special reagent for glucose has an exceptional value. This exists in phenyl-hydrazine, which, when added as a solution of the hydrochloride to a liquid containing glucose, to which sodium acetate has been also added, gives a yellow precipitate of phenyl-glucosazone. To apply the test, von Jaksch recommends that 50 c.c. of the suspected urine, previously freed from albumin, should be treated with 2 grammes of sodium acetate, and from 1 to 2 grammes of phenyl-hydrazine hydrochloride, and the liquid heated to 100°C. for half an hour; or 10 to 20 drops of phenyl-hydrazine and the same volume of 50 per cent. acetic acid may be employed. On cooling, the phenyl-glucosazone separates as an amorphous or crystalline precipitate of a yellow or brick-red colour. If amorphous, the precipitate should be dissolved in hot alcohol, and the solution diluted with water and boiled to expel the alcohol, when the glucosazone will be obtained in the form of characteristic yellow needles, melting at 205°C., nearly insoluble in cold water, more soluble in hot, moderately soluble in alcohol, and dissolved by glacial acetic acid to form a lævo-rotatory solution. According to von Jaksch, no sugar can be detected by this test in the urine of persons poisoned by arsenic, potash, or sulphuric acid; but the presence of sugar seems constant in the urine of those poisoned by carbonic oxide or other irrespirable gases.*

Instead of operating in the manner prescribed by von Jaksch, the phenyl-hydrazine test may be applied in the following simple manner, which is substantially that recommended by C. Schwartz (*Phar. Zeit.*, xxiii., 465): 10 c.c. measure of the urine is heated to boiling and treated with half its volume, or a sufficiency, of a 10 per cent. solution of neutral lead acetate. The liquid is boiled and filtered hot. Solution of caustic soda, in amount sufficient to redissolve the precipitate which first forms, is added to the filtrate, and then a little (as much as will lie on the point of a pen-knife) phenyl-hydrazine hydrochloride is dropped in. The liquid is then boiled for some minutes, and then strongly acidulated with acetic acid. In presence of much sugar an immediate yellow turbidity or precipitate will be formed, but if only minute traces be present a yellow coloration is first produced, which on cooling and standing changes to a turbidity. In all cases considerable time is required for the complete separation of the glucosazone, but the qualitative indication is readily and quickly obtained.

Unfortunately the phenyl-hydrazine test does not appear susceptible of being applied quantitatively, though, of course, the intensity of the reaction and the amount of precipitate afford a fair indication of the proportion of sugar present.

In all doubtful cases the indications furnished by the production of a turbidity or precipitate with the above test should be confirmed by obtaining the glucosazone in a crystallized form, examining it under the microscope, and, when possible, deter-

* Is it not possible that the traces of sugar apparently present in the urine of some persons in perfect health have their origin in the imperfect combustion of coal-gas?—A. H. A.

mining its melting point. I have found that it is readily dissolved by ether from its acidulated aqueous solutions. On separating and evaporating the ether the glucosazone can be dissolved in alcohol, and crystallized by adding water and evaporating, as already described.

As small a proportion as 0·05 per cent. of sugar can be positively detected in urine by the phenyl-hydrazine reaction. I have obtained distinct indications of sugar by means of it in samples of urine from apparently healthy persons.

Both dextrose and lævulose yield identically the same glucosazone. The only other constituents of urine which simulate the behaviour of glucose with phenyl-hydrazine are glycuronic acid and its compounds.*

Glycuronic acid is a syrupy liquid, miscible with alcohol or water. When the aqueous solution is boiled, evaporated, or even allowed to stand at the ordinary temperature, the acid loses the elements of water and yield the anhydride or lactone ($C_6H_8O_6$), which forms monoclinic tables or needles, having a sweet taste and melting at 167°. It is insoluble in alcohol, but dissolved by water to form a solution which is dextro-rotatory ($[\alpha]_D = +19{\cdot}25°$), prevents the precipitation of cupric solutions by alkalies, and powerfully reduces hot Fehling's solution, the cupric oxide reducing power being 98·8 compared with glucose as 100.

Glycuronic acid itself is dextro-rotatory ($[\alpha]_D = +35°$), but many of its compounds are lævo-rotatory. It reduces Fehling's solution on heating, and precipitates the metals from hot alkaline solutions of silver, mercury, and bismuth.

With phenyl-hydrazine, glycuronic acid forms a yellow crystalline compound, melting at 114 to 115°C.

When oxidized with bromine glycuronic acid yields saccharic acid, which can be again reduced to glycuronic acid by treatment with sodium amalgam.

Glycuronic acid is distinguished from glucose by not undergoing the alcoholic fermentation when treated with yeast. On the other hand, when fermented in presence of cheese and chalk it yields lactic and acetic acids.

Glycuronic acid occurs in the urine after the administration of morphine, chloroform, and certain other drugs. In one case recorded by H. H. Ashdown large amounts of glycuronic acid occurred in the urine of a healthy young man, which urine was not abnormal in volume or density.

No method of detecting glycuronic acid has yet been devised short of its actual isolation, and to effect this a large quantity of the urine is required. The method is described in an interesting paper by H. H. Ashdown (*Pharm. Jour.* [3], xx., 607).

Discussion.

The Chairman (Mr. Otto Hehner) invited discussion, and said that Mr. Allen had in the course of his paper afforded another proof of his indomitable industry in the

* Glycuronic acid contains $C_6H_{10}O_7$; or, $COH(CH.OH)_4.COOH$. The substance doubtless has its origin in the dextrose of the body, to which compound it is closely related. It was first obtained in the conjugated form of campho-glycuronic acid, in the urine of dogs to which camphor had been administered, and subsequently as uro-chloralic acid after the administration of chloral. It is remarkable for its tendency to form ethereal or glucosidal compounds when appropriate substances are introduced into the body. Traces of such compounds probably occur normally in urine, especially indoxyl- and skatoxyl-glycuronic acids, in addition to the combination with urea, having probably the constitution of uro-glycuronic acid, which is the ordinary form in which glycuronic acid exists in urine.

collection of facts, and had made an important addition to the literature on the subject of the paper.

Mr. G. Stillingfleet Johnson thought that Mr. Allen's paper was of especial value from a historical point of view. He had gathered together the observations of a number of foreign as well as English chemists on the subject, and had shown how difficult it was to get rid of an error when it once got into chemical literature and text-books. There could be no doubt that Brücke was wrong when he said that sugar was present in considerable quantities in normal human urine, and Bence Jones was wrong in supporting him in this country. But it was probable that the more delicate tests which now prevailed had enabled analysts to overcome these errors, and he thought present-day members of the profession ought not to be too hard upon their predecessors, who were not thus equipped. He thought that a test like the phenyl-hydrazine test was one of the utmost value. Not only did it apparently produce absolutely no reaction with any normal ingredients of the secretion of the human kidney, but it also appeared to produce no reaction with any of the numerous carbo-hydrates which were present, probably, in all urines, and which gave reactions with such tests as the alkaline copper test, the bichloride of mercury test, and a number of others. He did not lay very much stress upon Mr. Allen's observation that in some cases he had found sugar in urines, but he laid much more stress on the fact that he had not found sugar by those tests in other normal urines. The question was, What is a normal urine? This was simply reduced to the further question, Who is a healthy man? Everybody knew that a man might rise in the morning a healthy man, and that he might go to bed at night anything but a healthy man. He was quite sure that slight errors of diet—such, for instance, as taking a late dinner, or dining out—were sufficient to produce a temporary glycosuria, which was of no importance whatever. If the test were applied carefully to the urine of healthy individuals, it would be found in the long run to give practically negative results. He had no doubt that the reason why the idea that normal human urines were saccharine in character had existed so long was that the reducing reaction of creatinine had been mistaken for the reducing action of sugar, as Mr. Allen had pointed out. The Germans to this day under-estimated the reducing action of creatinine, which, he did not think, they put at one-eighth of its actual amount, and this he attributed to isomeric changes undergone by the creatinine in the act of isolation from the urine. In isolating this substance from urine, as could be seen by reference to his paper in the Royal Society's Proceedings, vol. xliii., he did not apply heat at a single stage of the process used. All the evaporations were done *in vacuo* over sulphuric acid, and no powerful chemical was used unless it was absolutely necessary; and the result was, as was pretty generally known, that the properties of the creatinine which he had isolated were completely different from those attributed to creatinine by other observers. Its reducing action far exceeded that of any creatinine previously obtained. Now that it was known that creatinine was a powerful reducing agent, and that a test like the phenyl-hydrazine one, which was capable of reacting with glucose in a human urine, existed, he hoped that the errors which were accidentally introduced so many years ago would at last be rectified, and that a clearer understanding on the matter would in future prevail.

Dr. James Edmunds said that physicians would be grateful to Mr. Allen for reinvestigating this subject of diabetic urines. In life insurance reports and in clinical work there was sometimes a difference of opinion as to how far the reducing action of certain urines depended upon the presence of small quantities of glucose, how far upon the presence of other reducing substances. He trusted that Mr. Allen would show them how to eliminate the non-glycosic reducing substances, and how then to proceed with sure steps to discriminate, and perhaps to quantitatively determine 0·1 per cent., or less, of diabetic sugar.

As to the grass-green coloration shown by Mr. Allen as due to hydrochloride of creatinine, he (Dr. Edmunds) had noted that the blue copper solution also became grass-green under the colorific action of yellow or orange matters in the urine. That this greening was due to colorific action as such, and apart altogether from any chemical reaction on the copper, was proved by simply crossing before the light two tubes, one containing such a urine, and the other containing the dilute Fehling. The same effect was seen by using tubes respectively containing ferric chloride and copper sulphate, each of a suitable depth of colour. And these solutions, if mixed, also gave the grass-green colour.

Another colour fallacy was apt to occur when urine containing sugar was boiled with caustic soda in order to throw out the earthy phosphates, or in titrating back after having added an excess of diabetic urine to the boiling alkaline copper solution. In this way small quantities of sugar might be caramelized, so as to give the filtrate a yellow or brownish tint, such as would at once give a grass-green or bronze-green colour with the Fehling's solution.

Sometimes there came out a muddy precipitate of a terra-cotta colour, which obstinately remained in suspension, until, by reabsorption of oxygen, it went back into a blue solution. This terra-cotta precipitate was probably a hydrated suboxide of copper, kept in suspension, like fine clay, by some urinary substances. But he would like to have Mr. Allen's opinion as to the cause of this reaction.

In hospitals the testing of urines should not be done in the wards. The urines, properly labelled, should be taken down to the pathological laboratory, and tested by a skilled chemist, from whom the students and house-surgeons would learn more than they now did by the objectionable and dangerous habit of keeping urinary reagents in each ward, for the use of students and nurses. He (Dr. Edmunds) once had a patient poisoned in one of his wards by drinking a corrosive acid from a ward-set of urinary reagents.

Mr. Hehner asked Mr. Allen whether he could give any information as to the actual quantity of residual sugar found after treatment with mercuric chloride.

Mr. Cassal wished to know whether there was any likelihood of a sensible loss of sugar by the previous preparation through which the urine passed. It seemed to him that this was very possible.

Mr. Allen, replying to Mr. Hehner, said that Pavy's solution gave perfectly definite and, he believed, true results for the estimation of sugar in urine, if it had been previously put through Mr. Johnson's process. The point on which he differed from Mr. Johnson was that he had not hitherto met with a sample which gave abso-

lutely no reduction by Pavy's test after going through Mr. Johnson's treatment. Although the amount was very small, down to 0·05 per cent., after treatment by Mr. Johnson's mercurial process, he had met with no instance of urine giving a wholly negative result. There was no difficulty in applying Pavy's solution to the estimation of small quantities of sugar, provided that too much of the copper solution was not used. If the results were checked by the production of the crystalline glucosazone by the phenyl-hydrazine reaction, there was a strong presumption of the existence of sugar. Caramelizing of glucose, no doubt, occurred when the Fehling test was applied in the ordinary way. The grass-green colour produced in the cold by creatinine on adding Fehling's solution was very characteristic. Analysts had been in the habit of regarding almost all reductions of Fehling's solution as due to sugar; but, as a matter of fact, there were hundreds of urines which gave a reduction which was not due to the presence of sugar. Whether the residual reduction after treatment with mercury was really due to sugar, as he was inclined to believe, he did not know with absolute certainty. The crystals of glucosazone obtained had been too small in amount in these cases for him to take the melting-point. If glycuronic acid were present, it might have been mistaken for sugar, as it gave a similar crystalline compound with phenyl-hydrazine. He did not quite see why one should be limited to processes involving no heat, in order to isolate creatinine, seeing that it was more convenient to employ heat, the precipitation being then effected in a few minutes, instead of several days. If it were found that creatinine could be extracted from urine in Mr. Johnson's way by means of heat, and that it had when so extracted a constant reducing power, which might be different from that of the substance obtained without heat, a method of estimating creatinine quantitatively could be based thereon. However, he was endeavouring to effect the ready determination of creatinine on another principle.

Mr. Hehner said he quite understood that a determination could be made as to how much urine would be necessary to decolourize a measured quantity of Pavy's solution. But did that afford a measure of the quantity of sugar present? The solution was here used under conditions differing from those prescribed by Dr. Pavy, and he (Mr. Hehner) did not notice that Mr. Allen employed any factor for correction.

Mr. Allen, regarding Mr. Hehner's observation that the introduction of large quantities of saline matters changed the reducing figure for Pavy's solution, said that he had ignored that.*

Mr. Cassal asked Mr. Allen whether there were any effects in the way of loss due to the application of the preparatory process.

Mr. Allen said that he adopted the plan of adding to urine a definite quantity of glucose, and had obtained an increase exactly corresponding to the glucose added.

* Since the paper was read, experiments, made specially with a view of ascertaining the influence of the zinc and sodium salts and the excess of ammonia used, have shown that the error from this cause may be neglected, at least when such small quantities of sugar as those in question are under consideration.—A. H. A.

The Recent Modifications of the Reichert-Meissl Method. C. Bunte. (*Chem. Zeit.*, 1894, xviii., 204-206.)—In defence of his modified Reichert-Meissl method, Kreis has maintained that an acid of definite concentration is essential to success (ANALYST, xviii., 166); it must be noted, however, that the specific gravity which he gives for an acid of 91·53 per cent.—viz., 1·825—is not in accord with the value which is assigned by Lunge and Naef to acid of this concentration, viz., 1·8298. Kreis also states (*loc. cit.*) that the correct concentration of the acid is indicated by the rapidity with which it hydrolyses butter-fat and margarine-fat.*

The author has used acids of both the above-quoted and of other specific gravities, and finds that with none of them is there sufficient constancy in the rapidity of hydrolysis to constitute a valid indication of the strength of the acid.

Pinette, Prager and Stern (ANALYST, xviii., 145) are correct in their statement that sulphurous acid is always produced during the hydrolysis. The following table gives the mean values obtained in experiments conducted with four acids of different specific gravities and with strict adherence to the prescription of Kreis:

Sp. Gr. of Acid.	Reichert-Meissl No.	Kreis's Reichert-Meissl No.
1·8215 ...	27·90 c.c. $\frac{N}{10}$ KOH ...	21·71 c.c. $\frac{N}{10}$ KOH
1·8252 ...	27·39 ,, ,, ...	25·04 ,, ,,
1·8298 ...	27·90 ,, ,, ...	26·35 ,, ,,
1·8393 ...	27·90 ,, ,, ...	32·33 ,, ,,

The duration of the process of hydrolysis was $\frac{1}{4}$ minute with the most concentrated acid, and 1$\frac{1}{4}$-1$\frac{1}{2}$ minute with the least concentrated, so that the number of c.c. of KOH used is larger the shorter the time of hydrolysis. The sulphurous acid in the distillate increased with the strength of the acid, being equivalent to from 1 to 4 c.c. $\frac{N}{10}$ KOH.

The influence of temperature was next studied, and was found to be very considerable; thus, with the same acid the number obtained was 28·82 c.c. $\frac{N}{10}$ KOH when the fat was "still warm," and 15·55 c.c. KOH when it was at 25° C., at the beginning of the hydrolysis.

It must be concluded that in Kreis's method only a partial hydrolysis, depending for its degree of completion on the strength of the acid and the temperature of the butter-fat is attained, the apparent agreement with the Reichert-Meissl numbers, observed in many cases, being procured by titrating sulphurous acid as volatile fatty acids.

Prager and Stern's suggestions (ANALYST, xviii., 145) the author sets aside as impracticable. Pinette (ANALYST, xviii., 167) obtains numbers which are too high, either because he heats too strongly or uses too strong an acid; his statement that he adds the acid to the molten fat furnishes no information as to the temperature, because, as is well known, butter-fat melts at 31-31·5° and solidifies at 19-20°.

Inasmuch as the author found that the larger the quantity of butter-fat taken for hydrolysis, the lower the number obtained, it was suspected that the water retained by the fat influenced the action of the sulphuric acid; but in no case was butter-fat found to retain more than 0·21 per cent. of water, a quantity too small to be accused

* In one place Kreis speaks of butter and margarine as being used in this test. Obviously, the varying water-content of these materials would introduce variation in the rapidity of hydrolysis by diluting the acid, and the respective fats must be meant.

of exerting the influence suggested. It is incidentally remarked that filtered butter-fat will give a constant Reichert-Meissl number for many weeks.

The following method is next set forth as the only one which will give constant results, agreeing sufficiently with the Reichert-Meissl numbers: About 5 grammes of the butter-fat are weighed into a litre Erlenmeyer flask, which is then placed in a drying oven and heated to 100° C. Ten c.c. of sulphuric acid (sp. gr. 1·8355) are added, the flask being swung to and fro the while; immersion in a water-bath at 30° to 32° for ten minutes follows, and then 150 c.c. of water are added with constant agitation. The liquid is immediately titrated with a concentrated permanganate solution, until the pink colour is permanent for a few seconds, and further treated as is customary in the Reichert-Meissl method.

Great stress is laid upon the specific gravity of the sulphuric acid, which must agree with that given to at least three places of decimals.

The following table comprises the figures which the author adduces in support of this process.

No.	Reichert-Meissl No. c.c. $\frac{N}{10}$ KOH	No. by above Method. c.c. $\frac{N}{10}$ KOH	H_2SO_4 Sp. Gr.	Permanganate. c.c.
1.	29·44	29·16	1·8355	3·6
2.	29·44	30·68	,,	3·6
3.	29·44	30·53	,,	3·6
4.	29·44	30·00	,,	4·0
5.	29·44	29·66	,,	4·0
6.	29·44	29·74	,,	4·0
7.	26·61	26·54	,,	4·0
8.	26·61	27·22	,,	4·0
9.	26·61	27·22	,,	4·0
10.	29·53	29·74	,,	3·6
11.	31·28	29·95	,,	4·0
12.	30·77	30·20	,,	3·9
13.	30·74	29·69	,,	4·0
14.	28·70	28·60	,,	4·0

The results of this investigation may be thus summarized:

(1) The modifications of the Reichert-Meissl method heretofore proposed are either unscientific or impracticable.

(2) The Reichert-Meissl method gives more closely concordant results than the method above prescribed.

(3) The temperature, the concentration of the acid, and the size of the reaction flask must vary between the very narrow limits named, in order that the sulphuric acid method may be at all successful. A. G. B.

The determination of Carbonic Anhydride in the presence of Soluble Sulphides. A. Wolkowicz. (*Zeit. Angew. Chem.*, 1894, 165; through *Chem. Zeit.*) In the ordinary process of determining carbonic anhydride in the presence of soluble

sulphides CO_2 and H_2S are evolved together, the latter absorbed in a tube of pumice soaked in copper sulphate and the former in a weighed potash bulb apparatus in the usual manner. This plan can be improved by adding cupric chloride to the contents of the evolution flask instead of using a copper sulphate absorption tube, the H_2S being thus retained as CuS, while the CO_2 escapes from the slightly acid solution. The process is particularly applicable for such substances as slag cement.

B. B.

REVIEWS.

THE SALE OF FOOD AND DRUGS ACTS, 1875 AND 1879. By T. C. H. HEDDERWICK, M.A. (Eyre and Spottiswoode. Price 5s.)

This little book, although avowedly written for practising lawyers and justices of the peace, ought immediately to find a place in the library of every public analyst. It contains, in a condensed and most intelligible form, every High Court judgment which affects the working of the Act, and from which the reader can see at a glance how the clauses and verbiage of the Acts have been interpreted by the judges. Of legal quibbles, and of judgments which show ignorance of chemical facts, there are not a few; but on the whole good sound common-sense prevails. Every page contains something of interest to the public analyst, who is bound, if he desire to remain on the list of the "efficients," to know the law as applied to every phase of the Act. Analysts would do well to study carefully the section on the "form of the certificate," and also the complications of the warranty question. We specially commend to their attention the admirable judgment of the late Lord Chief Justice Coleridge, who said: "This Act was passed with the object, not of punishing the seller, but of protecting the buyer, and of insuring, as far as it is possible to ensure, such a result that a person who buys an article of a particular description should get a genuine article, and one of which *contains the proper quantity of the different elements that an article of that description ought to contain.*" After such a decision, what becomes of the milks from starveling cows; of limits designed to pass exceptionally poor milks as genuine standards; and of the absurd contention emanating from Somerset House, that as long as an article has had nothing added to it, or abstracted from it, the law holds it to be genuine?

It is a pity that the little book does not also deal with the Margarine Act, the decisions concerning which can hardly be separated from those given under the Food Acts.

Appended to the book are notes on "Milk Adulteration," consisting of extracts from the evidence given before the Parliamentary Committee of 1872, mainly by the late Drs. Tidy and Voelcker. These tended to show that, in their opinion, genuine milk may contain as little as 10 per cent. of total solids, and that no milk with more than 10 per cent. ought to be condemned on analysis alone. Though the basis upon which these conclusions were founded has long been shown to be a fallacious

one, yet analysts would do well to make themselves thoroughly acquainted with the facts, or alleged facts, because after this book has come into the hands of defence solicitors, as it no doubt widely will, we shall hear a great deal more of the product of these wretched starveling cows and other "animated bundles of bones." O. H.

NATURE'S HYGIENE. By C. T. KINGZETT, F.I.C. London: Baillière, Tindall and Cox.

That a fourth edition of this book has been called for since its first publication in 1880 is a matter of congratulation to the author, and a proof that it has met with general acceptance by the class of readers for whom it was intended. It is especially suitable for those who have little or no knowledge of chemistry, and it gives a clear summary of each of the separate subjects which it embraces. These, as will be seen from a glance at the chapter headings, are far too wide to be more than lightly touched upon in the short space of five hundred pages. At the same time, the author has found room to notice many of the latest discoveries.

As in previous editions, the book is divided into two parts. The first, after an account of the general principles of chemistry, and especially of oxidation, deals somewhat more at length with sanitary subjects, such as water, sewage, bacteria, and disinfectants. In the second part, the various industries connected with the pine and eucalyptus are described, and an account is given of experiments made by the author to prove the disinfecting properties of oxidized terpenes.

The chapter on sewage is perhaps one of the best, and no clearer digest of the different methods of treatment could be desired. In this connection it may be mentioned that the statement on page 83, as to the probability of pathogenic organisms in sewer gas, is not altogether in agreement with recent experiments. In a report* on the subject to the London County Council Mr. Laws stated that he had found that the organisms in the gas issuing from sewers were in almost every case non-pathogenic, and that the two micro-organisms, *bacillus coli communis* and *micrococcus ureæ*—both of which must be present in abundance in sewage—were conspicuously absent from the gas. The immunity which sewermen enjoy from zymotic disease seems to point in the same direction, as does also the work carried out by Messrs. Carnelly and Haldance on the sewers of Westminster Palace and of Dundee.

In the section dealing with bacteria and their products the graft theory of disease is described at some length, but little mention is made of the at least as probable theory that the protozoa are the cause of certain diseases (*e.g.*, cancer) of which the bacterial origin cannot be proved. A short description of the different diseases and the micro-organisms associated with them is given in pp. 283-306; but the frequent mention of a well-known excellent disinfectant might have been spared in a work which professes to be of a purely scientific character. With the remarks of the author in the preface on the importance of having properly qualified chemists to do the work which is now often done by the incompetent, we cordially agree. C. A. M.

* Report to the Main Drainage Committee of the London County Council on Sewer Air Investigation, by J. Parry Laws, F.I.C.

MICRO-ORGANISMS IN WATER: THEIR SIGNIFICANCE, IDENTIFICATION AND REMOVAL. By PERCY FRANKLAND, Ph.D., B.Sc. (Lond.), F.R.S., and Mrs. PERCY FRANKLAND. London: Longmans, Green and Co. Price 16s. (net).

The bacteriological investigation of water has now assumed a highly-important place besides that of the usual chemical analysis, and as time progresses this is certain to become more and more the case. It is highly desirable, therefore, that every analyst engaged in the examination of drinking-water should possess an intimate acquaintance with the biological side of the question; and to those who are not *au fait* in this direction, the work before us will be found of the greatest possible assistance, whilst its appearance will be hailed with satisfaction by all earnest workers in this department. The names of the authors are in themselves a guarantee as to the ability and thoroughness which would be brought to bear upon any task of this nature which they might undertake, and it must be freely admitted that in this work they have fully maintained their reputation. In addition to the record of an enormous amount of original work, almost everything of importance which has appeared in English or foreign literature in relation to the question is referred to, and its source duly acknowledged; indeed, the reader cannot fail to be struck with the immense amount of literature which the authors have perused in the course of their investigations during the last ten years.

The work is divided into nine chapters and an appendix. The first two chapters treat on the operative details of biological work; and whilst these are given in a terse form all that is necessary is included, and the directions are stated in such an intelligible manner that no one should find the slightest difficulty in following them. Chapter III. describes the actual examination of water for micro-organisms, and contains important information as to the collection of samples, the general and particular appearance of colonies, etc. The next chapter, which is a very important one, treats on the purification of drinking-water and of sewage effluents by the various methods of filtration commonly in use. It not only shows the importance of bacteriological examinations in determining the amount of work which each individual construction of filter is capable of accomplishing, but also in ascertaining afterwards whether such filter is performing its work efficiently or otherwise. Quite a new light is thrown upon the beneficial effects of filtration; it is shown that these are not so much due to an alteration in the chemical constitution of the water as to the amount of micro-organisms eliminated. This is proved by a very extensive series of experiments undertaken by one of the authors, the results of which are both extremely interesting and surprising. Chapter VI. treats of the multiplication of the organisms in water under varying conditions; Chapter VII. on the detection of pathogenic species, and gives many remarkably ingenious processes which have been contrived to obviate the, in some cases, almost insuperable difficulties which attend their identification. Chapter IX. is devoted to a description of the action of light on bacteria; and lastly comes the Appendix, which is one of the most important divisions of the whole book; it contains a list, numbering upwards of two hundred, of all the micro-organisms which have been so far found in water, their microscopic appearance, their behaviour when cultivated in or on the several mediums, together with other details necessary for their identification.

The work is printed in bold, clear type, illustrated with engravings where necessary, neatly bound, and is provided with a good index. We can recommend it with every confidence as a work unique in character and absolutely indispensable to all who are engaged in the sanitary examination of water. W. J. S.